Epidemiology of Sheep Lice and Efficacy Evaluation of 60% Diazinon and 1% Ivermectin against *Bovicola ovis* in Sayint District, South Wollo, Ethiopia

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Abstract

Background

Sheep lice, caused by *Bovicola ovis* are very common in Ethiopia. It can result decreased production and reproduction, downgrading and rejection of skins in tannery industries. A cross-sectional and experimental study were conducted in Sayint District, South Wollo, Ethiopia which aims to determine the prevalence of sheep lice with the associated risk factors, identify the major sheep lice species and evaluate the efficacy of 60% Diazinon and 1% Ivermectin against *Bovicola ovis*. Study kebeles and animals were selected using purposive and multistage sampling techniques. Accordingly, a total of 232 sheep were sampled to estimate the prevalence of sheep lice. Fifteen sheep for in-vivo and 80 *Bovicola ovis* lice for in-vitro test were used for drug efficacy trial, by using completely randomized design.

Results

The overall prevalence of sheep lice in this study was 48%. Hair length (OR = 2, P = 0.00), body condition (OR = 1.9, P = 0.02), agro ecology (OR = 1.19, P = 0.00) and season (OR = 2, P = 0.01) were significantly (P < 0.05) associated with sheep lice infestation. The dominant sheep lice species in the study area were *Bovicola ovis* with prevalence of 83% and mixed infestation of 17% *Bovicola ovis* with *Linognathus ovillus*. In this study, the efficacy of 60% Diazinon and 1% Ivermectin against *Bovicola ovis* were 97% and 81%, respectively.

Conclusion

The overall prevalence of sheep lice infestation in the present study area was higher, in which *Bovicola ovis* was predominant and resistant against 1% Ivermectin. Therefore, tailor made intervention is required with a view to reducing the prevalence of sheep lice infestation and addressing drug resistance in the study area.

Background

Ectoparasite of sheep are very common and widely distributed in all agro-ecological zones in Ethiopia and sheep lice are considered as one of potential threats which pose a serious economic loss to the farmer, the tanning industry and the country as a whole [1, 2]. Skin problems caused by lice can result in mortality, decreased production and reproduction, downgrading and rejection of skins [3]. Genus *Linognathus* are obligate hematophagous ectoparasites of mammals and these ectoparasites harm the health of their hosts by blood sucking and this leads primarily to enormous losses of blood. Furthermore, the biting sites become super infected with bacteria and these wounds attract licking flies [4, 5]. *Bovicola ovis* is one of the most common lice found on sheep. They feed by chewing on the skin surface and surface debris and produce itching, irritation and possible hair loss. An allergic skin hypersensitivity

reaction to lice is another cause for “Cockle” in processed sheep skins [6]. In this regard, lice infestation in sheep cause cockle and it is the commonly reported disease in Ethiopia [7–9].

Control of sheep ectoparasites is currently an integration of sheep husbandry, farm management and insecticide use. Chemical control remains the most important and widely used strategy against most insect pests around the world. However, studies have shown that multiple resistance mechanisms in insects confer resistance to a range of insecticide classes [10]. For instance, the result of widespread chemical use has been the sequential development of resistance of *Bovicola ovis* [11]. Usually, resistance will be suspected through lack of efficacy during clinical use. However, lack of efficacy could also occur due to inadequate application of a product which leads to resistant ectoparasite species against acaricides [12].

To this end, there is a paucity of information on the epidemiology of sheep lice; the risk factors associated lice infestation in the study area and insecticide efficacy in Amhara region in general and in Sayint district of south Wollo zone in particular. Therefore, this study was initiated to determine the prevalence of sheep lice in the study area, to investigate the major risk factors associated with sheep lice infestation in the district, to identify the existing sheep lice species in the study area, to evaluate the efficacy of 60% Diazinon and 1% Ivermectin against sheep lice.

Methods

Study Area

The study was conducted in Sayint district which is found in the western part of south Wollo zone, Amhara National Regional State which is located 590 km, 659 km and 189 km from Addis Ababa, Bahir Dar and Dessie, respectively. The altitude of the district ranges from 500 to 4247 m.a.s.l. The area constitutes three agro climatic zones, highland (>2500 m.a.s.l), midland (1500-2500 m.a.s.l.) and lowland (<1500 m.a.s.l.), which comprises about 42.8%, 34.6%, 22.6% of the total area, respectively [13]. The average temperature of the district ranges from 4-40°C and annual rain fall ranges from 800 mm to 1000 mm [14]. The main rainy season in this area is between early June and the end of September, in addition a small rain occurs between early February and end of April [13]. Livestock rearing is the most important economic activity in which shoat and cattle are the major livestock species kept in the area. The total number of animals kept in the district were 141, 921 cattle, 222, 996 shoat, 23,982 equine, 143,872 poultry.

Study Animals

Local breed sheep and their crosses with Awassi of both sexes and all ages managed under extensive production system were the study animal.

Study Design

Cross-sectional study

A cross-sectional study was applied from October 2018 to April 2019 in order to estimate the prevalence of sheep lice and to identify the major lice species of sheep and determine associated risk factors in the district. Sex, age, hair length, body condition, housing management (whether the sheep is housed separately or with other animals; as separate and mixed), agro ecology (highland, midland and lowland) and season of sampling (wet and dry season) was recorded and documented along with the sample collection. Sheep were categorized into two age groups; young (< one year) and adult (> one year), as described by [15]. Age estimation was done by dentition as indicated in [16]. Body condition scores of sheep were determined as either poor or good according to [17].

Sampling Method

Preliminary data were sourced from the respective district of agricultural office to have lists of kebeles and list of sheep producing households in the district. Purposive sampling technique was employed to select the study district based on the availability of sheep. Two stage stratified sampling was applied and proportional samples from each kebele were drawn. Accordingly, 34 kebeles were stratified based on agro ecology (nine highlands, nine midland and sixteen lowland). Three kebeles were selected randomly representing the three agro ecologies. Hence, 102, 60 and 70 sheep were sampled from Yegodo, Yegoda and Ada, respectively. The selected kebeles were visited and sampled twice during the study period on October 2018 and January 2019 representing the wet and dry seasons, respectively.

Sample Size estimation

The sample size calculation used in this study was calculated according to [18], using an expected prevalence of 6.94% in Kutaber [19], a 95% confidence interval and a required absolute precision of 5%. In total 99 sheep were used for this study. However, the sample size was increased to 232 to enhance the precision.

Experimental study

Due to limited number of experimental unit, completely randomized design was used to evaluate the efficacy of 60% Diazinon and 1% Ivermectin against *Bovicola ovis* in local sheep. About 15 naturally *Bovicola ovis* infested (greater or equal to 100) local male sheep aged between two to four years that have moderate body condition with short and medium hair size and not recently treated with any acaricide were used for this study. Study animals were obtained from three willing local farmers. Before the commencement of the trial, study farmers were trained how to uniformly manage their animals by the investigator and separate houses for each group of sheep was prepared and cleaned. Lists of individual sheep identification number were made and lottery method of randomization was used to allocate into three groups having five sheep each, and followed for 28 days. After 15days of acclimatization period, by proper straining and handling of animal, first group were sprayed with 60% Diazinon, the second group

was given 1% Ivermectin injection subcutaneously as per manufacture's recommended dose and the third group was left untreated as a control. Both in-vivo and in-vitro efficacy trials were performed for Diazinon as per the protocols given by [20], while only in-vivo efficacy trial was conducted for Ivermectin as per [21]. Lice count was performed every 7 days for treatment and control groups and recorded starting from day 0 prior to treatment, days 7, 14, 21 and 28 according to [22]. 60% Diazinon contains an active ingredient of 600g/l Diazinon produced by Adamitulu pesticide processing share company, Addis Ababa, Ethiopia. 1% Ivermectin was manufactured by Bash Pharmaceutical Company, Khartoum, Sudan, and it was imported by Rang veterinary company, Addis Ababa, Ethiopia.

In-vivo trial: Each selected sheep in treatment and control group was visually examined and lice from individual animal was counted and recorded separately for 60% Diazinon and 1% Ivermectin for the in-vivo evaluation. The total lice count was applied by direct examination of the body with naked eyes. Lice from each sheep was counted using parting method, classifying the body into five parts: neck, shoulder, withers, flank and rump after marking 4 partings per site on both sides of the body [20, 22]. Thus, twenty sites on each side of the body were examined by parting the fleece about 10 cm and counting all live lice observed, so that, the total count from 40 sites constitutes the body count for each animal. The total lice count per animal was estimated by summation of the lice number at each site. Finally, lousicide activity was checked using arithmetic mean louse count for 60% Diazinon and 1% Ivermectin group separately along with a control group which was calculated according to [23]. Thus, mortality, 98-100% indicates susceptibility and less than 98% is suggestive of the existence of resistance [24].

In-vitro trial: sufficient live and motile lice were collected manually from naturally infested sheep that came into clinics and immediately taken into the laboratory. After species identification, 80 *Bovicola ovis* louse were randomly allocated into treatment and control groups. Each group contains 4 replicates having 10 lice placed on each petri dish as per the protocol provided by [25]. Then, 60% Diazinon was diluted in water according to the manufacturer's recommendation (1:1000) and lice in the treatment group were immersed completely in 0.5ml of this solution of 60% Diazinon for one min [26] and the control lice in the same amount of distilled water. After one minute, the solution soaked and dried using Whatman filter paper. Then, the vital signs in which lice exhibited after treatment was checked at 10, 30, 60, 120 minutes, as well as 6 and 12 hours of contact time under microscope [25].

Accordingly, recording of vital signs of lice in the treatment and control groups was held in each visiting time as described by [26]. For the calculation of mortality, highly stringent criteria was used and lice only judged as dead if they are in the categories 3 or 4 after 10, 30, 60 and 120 minutes as well as after 6 and 12 hours of contact time with 60% Diazinon [26]. The percentage mortality was calculated using a formula described by [27] as follows;

$$\% \text{ Mortality} = \frac{\text{Number of dead lice}}{\text{Total number of lice}} \times 100$$

Where the insecticidal effect of Diazinon was classified as: "strong" when mortality is >80%, "moderate" mortality 80–60%, " weak" mortality 60–40%, "little or no activity" mortality < 40%.

Data Collection

Clinical examination

Data were collected in two seasons (October and January) to observe the seasonal lice infestation variation between the late rainy and dry seasons of the area. After proper restraining, clinical examination of each sheep was done using multiple fleece partings in the opposite direction in which sheep’s hair or wool normally rests. The skin was inspected carefully with naked eye and palpated across all parts of the sheep for the presence of ectoparasites and gross lesions that could be suggestive for clinical form of parasitic infestation [28]. Sheep having the parasite or lesions like alopecia and itching was considered as positive.

Lice collection and identification

Careful examination of the animal body, especially neck, shoulder, breast, ribs, back, and flank and rump areas was performed to detect the presence of lice by parting the body hair. Lice were collected manually and added into labelled universal bottles with 70% Ethanol. Collected samples were dispatched to Bahir Dar Animal Health Diagnostics and Investigation Center for laboratory analysis. Lice identification was done using stereo microscope according to the descriptions of [29].

Data Management and Analysis

Raw data obtained during sample collection and laboratory identification were checked, coded, and entered into Microsoft Excel spread sheet by the principal investigator and then the raw data was exported to STATA version 13 for analysis. Both univariable and multivariable logistic regression was used to examine and quantify the association between lice infestation and explanatory variables. These significant variables ($P<0.05$) by univariable logistic regression were further evaluated by multivariable logistic regression to adjust the effect of confounding. Moreover, independent sample t-test was used to compare mean lice burden between treatment and control group

Results

Prevalence of Sheep Lice

The overall prevalence of sheep lice in the study area was 48.3% (112/232), with the highest prevalence of *Bovicola ovis* (83%) followed by mixed infestation of *Bovicola ovis* with *Linognathus ovillus* (17%). No sole infestation with *Linognathus ovillus* found by this study (Table 1).

Table 1. Overall and relative prevalence of sheep lice species in Sayint district

(N=232)		
Lice spp identified	Number positive (n)	Relative Prevalence (%)
<i>Bovicola ovis</i>	93	83.04
Mixed infestation	19	16.96
Total	112	100
Overall		48.3*

*= total positive divided by total population (N)

Risk factor analysis

Five potential risk factors were significantly ($P<0.05$) associated with sheep lice infestation using univariate logistic regression. These included hair length, housing management, body condition, agro ecology and season (Table 2). However, these potential risk factors were further evaluated by multivariable logistic regression.

Table 2. Univariable and multivariable logistic regression analyses of risk factors associated with sheep lice infestation in the study area

Variables	Categories	Univariable logistic regression Analyses			Multivariable logistic regression Analyses		
		multivariate analyses					
		OR	95% CI	P value	OR	95% CI	P value
Sex	Male	0.9	0.56-1.58	0.83			
	Female						
Age	Young	1.4	0.85-2.41	0.17			
	Adult						
Hair length	Short	2.3	1.41-4.06	0.00	2.18	1.2 - 3.9	0.00
	Medium						
House Mg't	Separate	0.4	0.24-0.76	0.00			
	Mixed						
Body condition	Poor	2.5	1.4-4.3	0.00	1.9	1.1 - 3.5	0.02
	Good						
Agro ecology	Highland	4.0	2.1-7.6	0.00	3.4	1.7 - 6.7	0.00
	Midland	5.0	2.5-10	0.00	4.4	2.1 - 9.1	0.00
	Lowland						
Season	Wet	1.8	1.0-3.0	0.02	2.0	1.1 - 3.6	0.01
	Dry						

Mg't=management

According to the final model, hair length, body condition, agro-ecology and season were significantly associated ($P<0.05$) with sheep lice infestation (Table 2). The odds of lice infestation in medium size hair coated sheep were 2.2 times higher as compared to sheep with short hair. Likewise, the odds of lice infestation was 1.9 times higher in poorly conditioned sheep as compared to well-conditioned ones. As compared to the lowland agro-climate, the odds of sheep lice infestation were 4.4 and 3.4 times higher in midland and highland agro-climates, respectively (Table 2).

Efficacy of 60% Diazinon and 1% Ivermectin

In-vitro efficacy test: based on the vital signs lice exhibit during the test, mortality was calculated. Hence, from lice treated with 60% Diazinon, 38 of them did not show any vital signs with 12 hours giving a mortality of 95%. All lice in the control group had survived during the observation period. The test acaricide has killed 2.5% of lice at 10 min, 7.5% at 30 min, and 10% at 60 min, 17.5% at 120 min, 82.5% at 360 min (6 hour) and 95% at 720 min (12 hour). There was significant ($p=0.05$) variation in percentage mortality of lice between 60% Diazinon treated and untreated group. The highest mortality was observed in the treatment group at 720 minutes (12 hour). The over all in vitro efficacy of 60% Diazinon was 95%. The in-vitro post treatment mean number of dead lice per minute of time is shown in Fig 1. The mean number of dead lice in the treated group had slightly increased from 30 to 120 min and consequently high mortality percentage was observed between 120 and 360 min, with the highest pick at 720 min Fig. 1. However, there was no any change in the untreated group throughout the study period.

In-vivo efficacy test: the overall in-vivo efficacy of Diazinon 60% was 99%. Though very slight infestation on days 7 and 14 post treatment, there was a significant ($p<0.001$) reduction in mean lice burden on treated sheep as compared to the untreated group. After the second round spray, on day 14, all sheep were found free of lice infestation while mean number of lice burden on untreated sheep was progressively increasing The mean number of lice burden reduction by 60%Diazinon among treated and untreated sheep before and after treatment is shown in Table 3. Before treatment, there was nearly similar mean lice burden on treated and untreated groups. Significant reduction of mean lice count was observed in treatment groups, while, a relatively high level of infestation was observed in untreated group Fig. 2.

Table 3. Mean lice count among treated and untreated group after application of 60% Diazinon

Treatment days	Mean number of lice count $\pm$ SD				
	Treated, N=5	Untreated, N= 5	t	df	P-value
Day 0	446 $\pm$ 283	403 $\pm$ 163	0.29	6	0.086
Day 7	9 $\pm$ 3	414 $\pm$ 317	-2.85	4	0.000
Day 14	15 $\pm$ 10	415 $\pm$ 131	-6.80	4	0.055
Day 21	0.00 $\pm$ 0.00	465 $\pm$ 178	-5.83	4	0.002
Day 28	0.00 $\pm$ 0.00	634 $\pm$ 224	-6.31	4	0.001

The in-vivo test showed that the efficacy of 1% Ivermectin was 81%. Before the commencement of the treatment, there was nearly similar mean lice count among treated and untreated groups. Mean lice count reduction by 1% Ivermectin injection was observed from day 0 to day 14, with a gradual increase from day 14 till the end of the experiment. A significant mean lice count reduction ($p=0.01$) was observed among treated and untreated groups (Fig. 3 Table 4).

Table 4. Mean lice burden on treated and untreated sheep after 1% Ivermectin injection Sayint district

Mean number of lice count $\pm$ SD					
Treatment day	Treated, N=5	Untreated, N= 5	t	df	P-value
Day 0	373 $\pm$ 33	403 $\pm$ 163	-.409	4.335	.111
Day 7	90 $\pm$ 39	414 $\pm$ 317	-2.275	4.120	.000
Day 14	70 $\pm$ 13	415 $\pm$ 131	-5.860	4.078	.065
Day 21	79 $\pm$ 8	465 $\pm$ 178	-4.837	4.015	.003
Day 28	108 $\pm$ 15	634 $\pm$ 224	-5.228	4.036	.001

Discussion

In the present study, the overall prevalence of sheep lice in the study area was 48.3% which is higher than other previous similar studies in Bale [30] (5.5%), Gamo [31] (4.6%), Afar region [32](11.6%), eastern Ethiopia [33] (21.4%), Tigray region [34] (1.3%), Kombolcha [35](28.5%) and in Bahir Dar [36](11.5%) and [37](3.8%). However, this report is found lower than [38] in Aba Jima district (83%) and in Gondar [39] who reported (57%). The possible reason for such high prevalence of lice in the study area includes poor management, feed scarcity and lack of quality veterinary services. The present result is consistent with [40] in Tiyo District (49%), and [41] in Arsi Highland areas (54%).

Bovicola ovis was the dominant lice species isolated by the present study (83%) followed by mixed infestation of *Bovicola ovis* with *Linognathus ovillus* (17%). This finding is consistent with the reports of [41] and [42] who reported 86% *Bovicola ovis* and 12% mixed infestation (*Bovicola ovis* with *Linognathus ovillus*) in Arsi. However, it is much higher than the reports of (28) and [42] who reported 29% and 73% *Bovicola ovis*, respectively. Similar report was also done in Wolmera district by [43] who reported 83.23% prevalence of *Bovicola ovis* in sheep.

In the present study There was a significant ($P<0.05$) association between sheep lice infestation and hair length. Those sheep with medium hair coat are more likely to be infested as compared to short ones. This finding is in lined with other previous reports done by [38, 34].

According to the present study, poor conditioned sheep were more likely to be infested with sheep lice as compared to well-conditioned counterparts, which agrees with the results of other studies [9, 36, 41, 44, 45] who reported poor body condition sheep were more affected by ectoparasite infestation than good body condition., Poor body condition can be exacerbated by itching and disturbance during grazing. This condition can compromise the nutritional status of sheep and leads to immune suppression. However, the present finding contradicts the findings of [38], who reported the prevalence of *Bovicola ovis* was higher in well-conditioned animals.

In the present study, sheep kept in midland (OR=4.4) and highland (OR=3.4) agro-climates were more likely to be infested by sheep lice as compared to lowland agro-climates. This finding is consistent with

[34, 45], but it disagrees with [2, 44], who reported higher prevalence of lice was recorded in Highlands followed by midland and lowland areas. The reason for higher prevalence in midland and highland might be associated with the temperature and moisture requirement of *Bovicola ovis* as well as shearing practice of farmers in the study area. In midland areas, temperature and moisture are conducive for lice to multiply. In highland areas, as the climate is very cold, farmers don't practice wool shearing to protect their sheep from cold stress. This practice could predispose sheep to lice infestation.

A significant higher lice infestation rate was observed during dry season as compared to wet season. This finding is found compatible with other previous reports [30, 46], while it disagrees with others [31, 44], where higher prevalence of sheep lice was recorded in wet season than dry season. The significant discrepancy between dry and wet season prevalence could be associated with feed scarcity that can result in poor body condition. During dry period, feed scarcity is common which exposes sheep to starvation and poor body condition that lead to immune suppression.

The efficacy of Diazinon 60% against *Bovicola ovis* was 99% and 95% for in-vivo and in-vitro, respectively. Hence, as per [27] and [24], the drug is effective for the treatment of *Bovicola ovis*. This result is found in line with previous studies [18, 47], who reported the in-vitro and in-vivo efficacy of Diazinon was 100%. However, it is found inconsistent with [48], who reported Diazinon was 82% effective.

The overall in-vivo efficacy of 1% Ivermectin was 81%, which is below the standard for in-vivo trials set limit (98-100%), suggestive of drug resistance. This result is far lower than the findings of [49], who reported that Ivermectin was 100% effective against sheep lice based on pour on formulations [19, 47, 50]. The probable reason why reduced efficacy of 1% Ivermectin injection against *Bovicola ovis* could be associated with prolonged and inappropriate use of the drug, the frequent application of 1% Ivermectin drug with the same brand without rotation with other insecticide classes. The importance of these presumed factors associated with drug resistance was stated by [19]. It is also evident that lack of insecticide class rotation and high treatment frequency increases macro-cyclic lactones resistance of parasites [51, 52].

Conclusion

The present study revealed that there was a higher prevalence of sheep lice infestation in the study area, where *Bovicola ovis* was the predominant lice species affecting sheep. A number of potential risk factors were tested to determine significant association with sheep lice infestation. Hair length, body condition, agro ecology and season were significantly associated with lice infestation in sheep. The in-vivo and in-vitro trials showed that 60% Diazinon was more effective to treat *Bovicola ovis*, where as 1% Ivermectin fails to treat sheep infested with *Bovicola ovis*. Hence, subsequent application of tailor made intervention towards the identified risk factors along with rational choice of drugs against sheep lice is warranted to reduce the prevalence of sheep lice infestation in the study area.

Abbreviations

AGRIS International System for Agricultural Science and Technology

CDM Center for Disease Control and Prevention

CVMP Committee for Medicinal Products for Veterinary Use

OR Odd Ratio

m.a.s.l Meter above sea level

Declarations

Ethics approval and consent to participate

Experiment trial was done with the farmer's animal and oral consent was made with the owners after getting ethical clearance paper from the Board of School Research Ethics Review Committee.

Availability of data and material

Not applicable

Consent of publication

Not applicable

Competing of interest

The authors declare that they have no competing interests.

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Authors' contribution

SL planning of the study, data collection and drafted the manuscript. MH participated in the planning of the study, revised the manuscript. HT participated in the planning of the study, analysed, interpreting and editing of the final manuscript. YF involve during planning of the study, interpreting the finding and editing of the final manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

In-vitro mean dead lice count after 60% diazinon treatment in sayint district

Figure 2

In-vivo mean lice burden of sheep treated with 60% Diazinon per different days of inspection

Figure 3

In vivo mean lice burden for 1% ivermectin per days of inspection in sayint district

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